NOTE TO THE FILE

BNF0014

February 22, 1996

Subject: Tomato with a Modified Fruit Ripening Phenotype (Line 35-1-N)

Keywords:
Tomato, *L. esculentum mill* var. *cerasiforme*, *S*-adenosylmethionine (SAM), *S*-adenosylmethionine hydrolase (SAMase) gene, *sam-k* gene, *E. coli* bacteriophage T3, Kanamycin resistance, *kan*^{r}, Neomycin phosphotransferase II (nptII)

Background

In a submission dated January 16, 1996, Agritope, Inc., provided FDA with its summary of the nutritional and safety assessment of a bioengineered tomato containing the gene encoding the enzyme *S*-adenosylmethionine hydrolase (SAMase). The firm started consulting with the agency regarding this product on September 14, 1994.

Intended Effect of the Genetic Modification

According to Agritope, the intended technical effect of the genetic modification of the large red cherry tomato *L. Esculentum mill* var. *cerasiforme* is modification of the ripening phenotype resulting in the production of a more physiologically mature fruit that is able to withstand the rigors of the current production and distribution system. Agritope accomplished this by inserting the gene encoding SAMase from the *E. coli* bacteriophage T3 (under a promoter that directs fruit-specific expression) into the tomato genome. SAMase degrades *S*-adenosylmethionine (SAM), which is the penultimate precursor in the ethylene biosynthetic pathway.

Introduced Genetic Material

Agritope used the *A. tumefaciens* T-DNA transformation system to introduce the *sam-k* gene (with a modified E8 gene promoter from tomato and the untranslated 3' region of the nopalin synthase gene (*nos*) from *A. tumefaciens*) and the *kan*^{r} gene from transposon Tn5 (with *nos* promoter and untranslated 3' regions) into the tomato genome. The *sam-k* gene was modified to contain a consensus eukaryotic translation initiation site by altering the nucleotide sequence surrounding the ATG start codon. The inserted DNA also contains sequences from the right and left border

regions of T-DNA from *A. tumefaciens*, lambda-*cos*, *E. coli* ori T and partial *trf*A operon sequences. Agritope used Southern analysis of initial transformant 35-1 and progeny to demonstrate the integration of the T-DNA at a single locus and the stability of the genetic insert over several generations.

Safety of Expressed Proteins

Two new proteins namely, SAMase and the *kan*^{r} gene product, neomycin phosphotransferase II (nptII) are expressed in the transgenic tomato line 35-1-N. The nptII enzyme is approved for use as a selectable marker in the production of transgenic tomatoes (21 CFR 173.170 and 573.130).

According to Agritope, stable integration of the *sam-k* transgene in the tomato results in the production of SAMase in a fruit specific and temporally regulated manner. Agritope used western analysis to demonstrate that SAMase is not expressed in plant tissues other than fruit. Further, Agritope used RNase protection assays to show that expression of the *sam-k* gene in the transgenic tomato fruit is transient. Agritope stated that, because SAMase is expressed in a developmentally regulated fashion, the amount of SAMase protein that would be expected in ripe fruit is minimal.

Agritope used western blot analysis to calculate the level of SAMase in fruit from the transgenic tomatoes at about 25.6 pg/μg or 0.0026% of the total protein found in ripening tomato fruit. Agritope also stated that although the deliberate use of SAMase in fresh food is new, the ubiquity of the protein in the digestive tract of human beings due to its association with coliphage T3 infections suggests that background exposures to this protein are ongoing.

Agritope stated that neither the mode of action of SAMase, nor the by-products of the reaction catalyzed by SAMase, raise any safety concern. Database searches did not demonstrate any homology to any protein (and thus to any toxin or allergen) in the three major protein databases (University of Geneva protein sequence data bank (Swiss-Prot 31), the Protein Identification Resource sequence data bank (PIR26) and the GENESEQ Patented amino acid sequence data bank (A-Gene Seq. 21).

Agritope also concluded that consumption of SAMase in tomato fruit will not raise any allergenicity concerns because the enzyme does not have characteristics commonly attributed to food allergens; Agritope stated that SAMase 1) is not

glycosylated as it lacks the necessary sequence information needed to transport the protein to the subcellular compartments where glycosylation takes place, 2) is rapidly inactivated by incubation under gastric conditions, 3) is not heat stable, and 4) will be found at extremely low concentrations in ripe fruit (less that 0.65 μg/fruit).

Compositional Analysis: Nutrients and Endogenous Toxicants

Agritope concluded that line 35-1-N differs from the non-transformed parental line only in the presence of nptII and SAMase and their intended technical effects. Agritope stated that fruit derived from 35-1-N did not differ from fruit derived from the non-transgenic controls in pH, soluble solids or titratable acidity. Agritope also analyzed for Vitamins A and C, calcium, iron, sodium, dietary fiber, simple sugars, total protein, and the relative proportion of the individual amino acids, and found that tomato fruits from 35-1-N did not differ from their non-transformed counterparts in any of these constituents. In addition, Agritope stated that the values obtained for these parameters in both the transgenic and non-transgenic tomatoes fall well within the range normally reported for fresh tomatoes. Finally, Agritope stated that fruits derived from 35-1-N have tomatine levels that are comparable to those found in tomatoes derived from the non-transgenic parental line.

Conclusions

Agritope has concluded that tomatoes from transformed line 35-1-N are safe as food and do not differ significantly from traditionally developed tomatoes except for the intended technical effects of the *sam-k* and *kan*^{r} genes. At this time, based on Agritope's description of its data and analyses, the Agency considers Agritope's consultation on transgenic tomato line 35-1-N to be complete.

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